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An examination of the complex ecological role of tall fescue in grassland restoration

by

Karin Joanne Jokela

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Ecology and Evolutionary Biology

Program of Study Committee:
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ABSTRACT

Tall fescue (*Schedonorus arundinaceus* (Schreb.), an exotic-invasive, cool-season grass has invaded millions of acres of grassland in the United States. Tall fescue's dominance can be attributed, in part, to a fungal endophyte (*Epichloë coenophiala*) that confers fitness benefits to the grass, and which lives symbiotically within the grass host. With invasion into native grasslands, tall fescue alters litter production, changes composition and structure of vegetation, and modifies fire behavior. This thesis presents results from two studies on the complex role of tall fescue in Iowa grasslands. In the first study, I investigated how grassland management with fire and grazing influences tall fescue cover and litter dynamics. From 2012-2014, three fire and grazing treatments were applied to pastures with a mixture of tall fescue and other native and exotic plants in the Grand River Grasslands of Iowa and Missouri. We hypothesized that the fire-grazing interaction (a.k.a. patch-burn grazing) would reduce tall fescue cover more than fire or grazing treatments applied independently. We also hypothesized that because tall fescue may impede fire movement across the landscape, litter depth and woody plant cover would be positively correlated with the presence of tall fescue following use of this management technique. Over the three years of the study, tall fescue cover was not reduced as a result of the treatments, and there was only a weak positive correlation between tall fescue and litter cover. No correlation was found between tall fescue and woody plant cover. Years-since-fire had the greatest effect on litter dynamics – regardless of tall fescue abundance at the site - and the patch-burn grazing treatment had the most heterogeneous litter depths within each year. Our findings suggest that patch-burn grazing can benefit livestock producers and wildlife in fescue-invaded pastures, but it is not sufficient to reduce tall fescue cover. In the second experiment, we examined how invasive,

exotic grasses like tall fescue may be contributing to the decline of grassland butterflies through alterations in forage quality. Alkaloids produced by the endophyte are known to be toxic to some foliar-feeding pest insects, but effects of the endophyte on non-pest insects such as butterflies are relatively unknown. We examined growth and survival parameters of tawny-edged skippers (*Polites themistocles*) that were reared on endophyte-infected tall fescue (E+), endophyte-free tall fescue (E-), and Kentucky bluegrass (KBG). Results showed that the endophyte did not affect growth and survival of larvae compared to uninfected tall fescue, even though significant amounts of loline alkaloids (average 740 ppm) were measured in endophyte-infected plant material. Larvae feeding on KBG grew faster with greater survival rates than larvae on both tall fescue treatments. These results confirm that tall fescue invasion and dominance may be deteriorating the quality of grassland habitats for native pollinators; however, this effect does not appear to be linked to endophyte infection.

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CHAPTER 1. GENERAL INTRODUCTION

Natural grasslands are one of the most imperiled ecosystems in North America. More than 80% of grasslands have been lost since the mid-1800s, primarily due to conversion of the land for row crop agriculture (Samson and Knopf 1994). Those that were not plowed were often seeded to exotic forage pastures or developed for urban and industrial use. Exotic-invasive grass species have drastically altered the ecological processes that once occurred in the tallgrass prairie (D'Antonio and Vitousek 1992, Brooks et al. 2004), and understanding their impacts on communities and landscapes is vital to preserving some of the biodiversity and ecological processes that once flourished in North America.

One of the most ecologically- and agriculturally-influential exotic grasses in North America is tall fescue (*Schedonorus arundinaceus* (Schreb.)). This Eurasion, cool-season grass is now the most common grass species in the United States (Hill et al. 1991), and it is used globally for turfgrass and perennial forage for livestock (Fribourg et al. 1991, Rudgers and Clay 2007). While intentionally introduced in some areas for pasture improvement, parks and lawns, and roadside stabilization programs, tall fescue has also invaded about 14 million ha (35 million acres) of land in the eastern U.S. where it is considered undesirable (Ball et al. 1993). Tall fescue's dominance in the landscape can be attributed, in part, to a fungal endophyte (*Epichloë coenophiala*) (Morgan-Jones & Gams). that lives symbiotically and asymptomatically within the grass host. Fungal endophytes are ubiquitous in plants with varying host-endophyte interactions (Saikkonen et al. 1998, 2006); however, this particular grass-endophyte symbiosis has the potential to confer many fitness advantages to its host, including enhanced tillering and biomass production (Hill et al. 1991), improved resistance to

drought (Malinowski and Belesky 2000), increased seed production and germination rates (Clay 1987), and resistance to herbivores via toxic alkaloids (Clay and Schardl 2002, Cheplick and Faeth 2009).

Much of our knowledge about the anti-herbivore effects of alkaloids has been anchored in livestock health and biocontrol for generalist pest insects. It is now well known that fungal alkaloids (primarily ergot alkaloids) can cause reduced growth, conception, milk production, and intolerance to heat in cattle, horses and sheep that consume endophyte-infected agronomic grasses (Prestidge 1993, Hoveland 1993, Roberts and Andrae 2004, Young et al. 2013); similarly, there is much evidence that endophyte-produced alkaloids (primarily lolines) are toxic to invertebrate herbivore pests (Clay 1989, Siegel et al. 1990, Leuchtmann 1992, Latch 1993, Breen 1994, Wilkinson et al. 2000).

More recently, ecologists have been studying the effects of endophyte-infected tall fescue on grassland biodiversity and ecosystem processes, and the impacts have been slightly more equivocal (Spyreas et al. 2001, Matthews and Clay 2001, Rudgers et al. 2004, Rudgers and Clay 2007, 2008, Crawford et al. 2010, Iqbal et al. 2012). It seems the challenges of managing endophyte-infected tall fescue have become increasingly complex as we learn more about the positive and negative impacts it has on communities and landscapes. Nevertheless, these studies are invaluable if our aim is to restore biodiversity and ecosystem services while maintaining agricultural productivity in North American grasslands.

Research in this thesis addresses two novel aspects of tall fescue ecology. First we explore the ways in which tall fescue influences (and is influenced by) patch-burn grazing for tallgrass prairie restoration and, second, we investigate the effects of tall fescue on growth and development of a native tallgrass prairie invertebrates.

Historically, large herds of herbivores (e.g. bison) migrated throughout the prairie, foraging in areas with tender regrowth following recent fires. This fire-grazing interaction has given rise to the grassland management technique called patch-burn grazing that restores the natural fire-grazing interaction under which tallgrass prairie systems evolved (Axelrod 1985, Fuhlendorf et al. 2009). This management technique provides the basis for our experimental approach in the first study. We know that invasive grass species are altering ecological processes (D'Antonio and Vitousek 1992), and are thus creating significant challenges for habitat managers. To our knowledge, no one has tested whether fire and grazing (via the patch-burn grazing system) can control tall fescue invasion. Managing tall fescue with fire and grazing could be a more preferable option than using the broad-spectrum herbicide, glyphosate, that can be expensive and potentially pose risks to non-target species. If patch-burn grazing operates as theory predicts (Fuhlendorf et al. 2009, McGranahan et al. 2013), we would expect that it would set back the spread of tall fescue by reducing its competitive edge; however, there are some unique, robust qualities of tall fescue that could alter the fire-grazing interaction rendering the technique to be less efficacious as a control agent. For example, tall fescue greens up early in the growing season when prescribed fire is typically applied. As a result of the live (and moist) fuel-bed, fires have the potential to be incomplete in areas with higher tall fescue densities (McGranahan et al. 2012, 2013). It is unclear whether incomplete, or "patchy" burns would draw in herbivores with the same intensity as complete burns (Archibald et al. 2005). There may be cascading effects that result from the tall fescue and fire-grazing interaction; for instance, litter production could increase in areas with abundant tall fescue cover through several mechanisms: endophyte-infected tall fescue is competitive and produces large amounts of biomass (Clay 1987),

endophyte-infected litter may decompose more slowly due to reduced decomposer activity (Lemons et al. 2005, Mayer et al. 2005, Siegrist et al. 2010), and/or litter may accumulate as a result of incomplete burns (McGranahan et al. 2013). The goal of our research was to investigate these possible outcomes, to better inform future restoration management decisions and yield successful results.

Tall fescue dominance in grasslands is also potentially of great negative consequence to native invertebrates, particularly when endophyte-associated alkaloids are present. In experimental plots, endophyte-infected tall fescue has been shown to: 1) reduce growth and survival of aboveground herbivores and seed predators, 2) restructure belowground herbivore and detritivore invertebrate communities, and 3) reduce species abundance and richness of natural enemies (e.g. spiders) in arthropod communities (Rudgers and Clay 2007). Still, not enough is known about the impact of the tall fescue-endophyte symbiosis on food web dynamics in natural ecosystems. Therefore, in our second study, we tested the effects of endophyte-infected tall fescue (E+), endophyte-free tall fescue (E-), and Kentucky bluegrass (*Poa pratensis*) on the growth and development of a grassland-dependent butterfly species: tawny-edged skippers (*Polites themistocles*). The larvae of this butterfly species are considered grass-eating generalists, and their exact host species is unknown. Interestingly, despite their apparent flexibility in diet, many populations of grass skipper species in the family Hesperiidae are in severe decline for unknown reasons (Swengel et al. 2011, Swengel and Swengel 2013). We used tawny-edged skippers as a surrogate species to test whether endophyte-associated alkaloids were negatively affecting skipper development and survival rates. The goal of this research was to improve scientific understanding of grass-endophyte ecology and butterfly conservation in exotic-grass invaded grassland ecosystems.

Both studies presented here were conducted at the Grand River Grasslands of Ringgold County, Iowa, and Harrison County, Missouri - an area over 28,000 ha in size and comprised of a matrix of fragmented grassland patches, woodlands, and row crop agriculture. Exotic forage grasses dominate the majority of the grasslands in the region, though there are a few pockets of remnant tallgrass prairie that host a broad suite of plant and animal diversity (Delaney 2014). Due to the fragmented nature of this primarily agricultural landscape, results from this region may be especially relevant to managers in other similarly fragmented parts of the tallgrass prairie.

Thesis organization

This thesis is composed of two chapters written for publication in scientific journals. Chapter 1 is a general introduction to the research presented in Chapters 2 and 3. Chapter 2 is a summary of the research I conducted as part of a grant funded by the Leopold Center for Sustainable Agriculture. As part of this project, I assessed the effects of tall fescue on grassland litter dynamics under fire and grazing treatments. Chapter 3 is an article that was recently accepted for publication in the journal *Environmental Entomology*. This original research investigated the effects of endophyte-infected tall fescue on the growth and survival of tawny-edged skippers (*Polites themistocles*), a native, grass-eating butterfly species. Chapter 4 provides a general conclusion to this research with thoughts on directions for future research.

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CHAPTER 2. EFFECTS OF TALL FESCUE ON GRASSLAND LITTER DYNAMICS UNDER FIRE AND GRAZING

A paper to be submitted to *Rangeland Ecology & Management*

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Abstract

In the United States, the exotic-invasive grass tall fescue, (*Schedonorus arundinaceus* (Schreb.)), has presented major challenges for grassland restoration. The grass hosts a fungal endophyte that can be toxic to herbivores, and can also decrease plant community diversity and alter litter dynamics. Litter depth in grassland habitat is an important component for grassland obligate bird and insect species. We hypothesized that patch-burn grazing would reduce tall fescue cover more than fire or grazing treatments applied independently. We also hypothesized that because tall fescue may impede fire movement across the landscape, litter depth and woody plant cover would be positively correlated with the presence of tall fescue following use of this management technique. Finally, we hypothesized that nitrogen in litter would be higher in recently burned areas. From 2012-2014, three treatments were applied to pastures with a mixture of tall fescue and other native and exotic plants in the Grand River Grasslands of Iowa and Missouri. The treatments included: 1) patch-burn grazing, where one-third of the pasture was burned each year on a rotating basis, and cattle had free access throughout the pasture; 2) graze-and-burn, where the entire pasture was burned once every

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three years and cattle grazed freely throughout the pasture; and 3) burn-only, in which the entire pasture was burned once every three years without any grazing. Over the three years of the study, tall fescue cover was not reduced as a result of the treatments, and there was only a weak positive correlation between tall fescue and litter cover. No correlation was found between tall fescue and woody plant cover. There was no effect of years-since-fire on nitrogen levels in the litter. Years-since-fire had the greatest effect on litter dynamics – regardless of tall fescue abundance at the site - and the patch-burn grazing treatment had the most heterogeneous litter depths within each year. Our findings suggest that patch-burn grazing can benefit livestock producers and wildlife in fescue-invaded pastures, but it is not sufficient to reduce tall fescue cover.

Introduction

Grasslands cover between 20-40% of the earth's surface, but are one of the most threatened ecosystems globally (White et al. 2000, Hoekstra et al. 2005). Biodiversity in these systems is in decline (Wilsey et al., 2009) and many of the remaining grasslands are now novel communities that are severely degraded by exotic species, altered fire regimes and continuous livestock grazing (Hobbs et al., 2006; Parr et al., 2014). In a world where agriculture and conservation are increasingly in competition for space and resources, it is imperative that land managers attempt to accommodate multiple agendas by restoring the diversity and function of native grasslands while maintaining agricultural productivity.

Recently, researchers and land managers have experimented with a new grassland restoration approach that focuses on restoring natural, ecological processes rather than reconstructing specific prairie communities. The technique, referred to as patch-burn grazing,

has the potential to enhance biodiversity and habitat heterogeneity while simultaneously benefiting livestock producers (Fuhlendorf et al. 2004, Fuhlendorf et al. 2009, Allred et al. 2011, Weir et al. 2013). Patch-burn grazing integrates two historic, natural disturbances that have long been decoupled in grassland management: grazing and fire. When fire is applied in discrete patches across a grassland landscape, herbivores tend to spend more time grazing on the tender regrowth of a recently burned area rather than areas with greater time since fire (Coppock and Detling 1986, Vinton et al. 1993, Wilsey 1996, Coppedge and Shaw 1998, Archibald et al. 2005). In fact, the fire-grazing interaction (aka pyric herbivory) is known to have a unique disturbance effect that is different than if fire or grazing had been applied alone (Archibald et al., 2005; Fuhlendorf et al., 2009). The interaction creates spatial heterogeneity in vegetation structure and fuel loads (Fuhlendorf and Engle 2004, Churchwell et al. 2008, Engle et al. 2008) and can potentially be used to manage invasive plant species (Cummings et al. 2007) and ecosystem processes by altering soil nutrient availability (Anderson et al. 2006). When applying patch-burn grazing at a pasture scale, one discrete patch of the pasture is burned each year, such that the whole pasture is burned over several years. Herbivores, most often cattle, are allowed to graze freely throughout the pasture without internal fencing, but given their preference for recently burned areas, the pasture is grazed unevenly throughout the season. While burned patches receive intense grazing pressure, unburned patches tend to "stockpile" plant biomass and litter, which, in turn provides fuel for future burns (Fuhlendorf & Engle, 2004). Manipulating fire and grazing in this way could be a valuable strategy for managing agricultural production and conservation of wildlife habitat.

Many agricultural grasslands are invaded with exotic-invasive grass species such as tall fescue (*Schedonorus arundinaceus* (Schreb.)) and patch-burn grazing has the potential to serve as a tool to manage invasive species. In a burned patch, grazers are less discriminating between plant species, and thus, they are more likely to consume forage evenly. As a result, they put more grazing pressure on invasive grass species that they may have avoided in conventionally-managed pastures. While patch-burn grazing may not eliminate non-native grass species, increased animal consumption could reduce their dominance in the plant community over time.

In the Grand River Grasslands (GRG) of southern Iowa and northern Missouri (Figure 1), tall fescue is one of the biggest threats to one of the best-known tallgrass prairie restoration opportunities (Missouri Department of Conservation 2005, McGranahan 2008). Although used widely as a seeded perennial forage for beef cattle, tall fescue also has invaded about 14 million ha (35 million acres) of land in the eastern U.S. where it is considered undesirable (Fribourg et al. 1991). Several beneficial traits (primarily forage yield and hardiness) are offset by several negative impacts to agriculture and natural resources. Tall fescue can host a fungal endophyte (*Epichloë coenophiala*) that produces toxic ergot alkaloids which cause symptoms in beef cattle commonly known as fescue toxicosis. Toxicosis symptoms include reduced blood flow to the extremities resulting in elevated body temperature, reduced milk production, reduced weight gain, reduced calving rate and shaggy hair coats (Roberts and Andrae, 2004). Tall fescue presence has been shown to coincide with reduced native plant species richness and insect biodiversity (Clay & Holah, 1999; Madison et al., 2001; Rudgers et al., 2004, 2005). With invasion into native grasslands, tall fescue has the potential to alter litter production and decomposition, change composition and structure

of vegetation, and modify fire behavior (McGranahan, 2012). The altered habitat reduces the value for a variety of wildlife species, including butterflies (Shepherd 2005) and grassland birds (Lyons et al. 2015).

Tall fescue management in prairie restoration has relied heavily on herbicides, which can potentially harm wildflowers and native cool-season grasses at the time of application (Washburn et al. 2000, Madison et al. 2001, Ruffner and Barnes 2010). Several studies have demonstrated that natural processes such as fire and grazing can set back invasive species in grasslands (DiTomaso et al. 2006, Cummings et al 2007, Rinella and Hileman 2009, Kessler et al. 2015), while others have shown that fire can encourage exotic species or have an equivocal effect on their dominance (MacDougall and Turkington 2005, Alba et al. 2015). Much less is known about the impact that the fire-grazing interaction has on the dominance of invasive species like tall fescue. Our objective in this study was to quantify the extent to which patch-burn grazing management interacts with tall fescue invasion to influence native warm-season grass communities, by investigating litter dynamics and litter nutrient retention. One of our hypotheses was that patch-burn grazing management would, over time, reduce tall fescue cover. In contrast, we expected that fescue cover would remain unchanged in treatments where fire and grazing were not combined. Assuming the patch-burn grazing management would behave as theory predicts, we also expected fuel loads to accumulate (i.e. "stockpile") in unburned patches of the patch-burn graze treatment. Litter depth and litter mass would thus be expected to increase with time since fire. These hypotheses support the patch-burn grazing model of increased structural heterogeneity.

Invasive species may affect habitat parameters such as plant litter quality, which can vary depending on a range of tissue components including carbon, nitrogen, phosphorus,

lignin and secondary compounds such as endophyte-associated alkaloids; the specific composition of these components can influence how the litter decomposes (Hobbie 1992, Omacini et al. 2004, Siegrist et al. 2010). Furthermore, leaf and litter tissue quality is affected by environmental conditions such as precipitation and fire and grazing management (Semmartin et al. 2004, Garibaldi et al. 2007). In this study, we sampled carbon and nitrogen dynamics in plant litter to determine the impact that tall fescue has on ecosystem processes, and to test whether there are differences in nutrient dynamics across treatments. Very little is known about carbon and nitrogen dynamics in aboveground tall fescue litter, but there is evidence suggesting nitrogen concentration in plants varies significantly by species (King et al. 2004, An et al. 2005). Thus, it is reasonable to assume that tall fescue dominance within grassland systems will contribute to overall changes in nutrient cycling. Furthermore, Anderson et al. (2006) showed that patch-burn grazing management could result in a pulse of soil nitrogen availability in the burn patch followed by intense grazing. We hypothesized that nitrogen in litter would be higher in recently burned areas.

The presence of exotic species such as tall fescue can cause unique challenges for grassland restoration efforts – partly because their novel effects on the ecosystem are still poorly understood. Several studies have reported that exotic grasses increase fire potential by altering the fuel-bed, thus creating a positive feedback cycle for more extensive invasion (D'Antonio and Vitousek 1992, Brooks et al. 2004). In contrast, through several mechanisms, tall fescue has been predicted to behave differently when subjected to fire. McGranahan et al. (2012, 2013) argue that tall fescue might have the opposite effect on fuel-loads because it introduces a moisture-rich, live fuel-bed when the remainder of the native plant community is dormant and relatively dry. Such a high-moisture fuel-bed would likely

reduce the intensity and the spread of fire in these systems. Thus, pastures with large proportions of tall fescue cover may not burn as uniformly, which could alter the intensity and evenness with which cattle graze in the burned patch. Given these complexities, we also put forth an alternative set of hypotheses about the ways in which tall fescue might interact with patch-burn grazing management. First, we hypothesized that litter depth would be greater in areas with high abundance of tall fescue cover. Second, incomplete burning and patchy grazing caused by tall fescue cover could facilitate woody plant invasion because these areas would not be subjected to intense disturbance, and soil moisture would remain more constant under a deeper litter layer. Therefore, our third hypothesis was that tall fescue cover and woody plant cover would be positively correlated. All of these hypotheses need to be evaluated in light of the tall fescue-fungal endophyte symbiosis. Systemic endophytes from the family Clavicipitaceae are predicted to retard decomposition of litter by altering its abundance and quality (chemical composition), as well as impacting decomposer organisms and their communities (Matthews and Clay 2001, Omacini et al. 2004, Lemons et al. 2005, Mayer et al. 2005, Rasmussen et al. 2007, Siegrist et al. 2010, Saikkonen et al. 2015). Tall fescue tillers sampled in the Grand River Grasslands have an endophyte infection frequency averaging between 60 and 80% (unpublished data). We did not measure endophyte presence or alkaloid levels in our litter samples, but these traits could play a role in litter persistence. Therefore, several mechanisms may operate by which litter depth is altered in patches dominated by tall fescue. This litter layer is important in controlling the way fire moves across the landscape and which may in turn affect nutrient retention and plant and insect community responses across the landscape. Our research evaluated these possible differential effects of tall fescue in native grasslands.

Methods

Fire and grazing treatments

We applied grazing and burning treatments to pastures with a mixture of native and exotic plants, including tall fescue, in the Grand River Grasslands of Iowa and Missouri, USA from 2012 to 2014. The treatments included: 1) patch-burn grazing (PBG) and 2) graze-and-burn (GAB), and 3) burn-only (BO) and each treatment was replicated on four independent pastures each year. In PBG pastures, a discrete one-third patch of a pasture was burned each year, and cattle were allowed to freely graze between recently burned patches and areas with more forage that have recovered from fire and grazing in previous years. Each year, a different one-third patch of the pasture was burned, which, according to previous studies (Fuhlendorf et al. 2009, Allred et al. 2011, 2014) should result in cattle following fire around the landscape. GAB represents a traditional strategy of using both fire and grazing by burning the entire pasture every third year. BO pastures were not grazed, and they were burned once every three years on the same schedule as the GAB pastures. The average pasture size was 65 acres. Stocking rate was moderate and averaged approximately 1.0 AUM's (animal unit months) per acre. Cattle were mature cows or replacement heifers of the Angus breed. Grazing was seasonal with cattle going onto pastures typically around mid-May and coming off of pastures in mid-October. Prescribed burns were conducted annually in March or April.

Litter dynamics

We assessed litter dynamics and litter nutrient content as a function of tall fescue cover for all three treatments (patch-burn graze, graze-and-burn and burn-only pastures). Burn-only pastures served as a comparison treatment because tall fescue presence was

minimal (approximately 12 percent) in most of those pastures compared to approximately 50 percent tall fescue cover in the grazed pastures.

Tall fescue cover, litter depth, and litter mass were assessed during the fall after the first frost, but before snow accumulation of each year by using established plant community transects (two per patch for a total of 6 per pasture). The length of each transect was established to sample the maximum extent of the patch and varied among patches from 100-300 m. Transect locations were mapped using GIS software (ArcMap 9.3, ESRI 2009). Within each patch, thirteen 0.5-m^2 quadrats were placed at equal intervals along the two transects. Within each quadrat, we estimated plant cover by functional group (warm season grass, cool season grass, tall fescue cover, forbs, legumes, sedges, woody cover litter cover) using an 8-point scale (0%, 1%, 3%, 16%, 38%, 63%, 85%, 96%) per Daubenmire (1959). In addition, litter depth was measured in the center of each quadrat to the nearest centimeter and litter biomass estimated systematically within each quadrat. Litter biomass (fuel) in each quadrat was assessed visually and categorized into a class of 0, 1, 2, or 3, with 0 and 3 representing the lowest and highest litter mass Twidwell et al. (2009), respectively (a minor variation of this approach was used in 2012, when pastures were measured on a 5-point scale of 0 – 4).

After measuring of depth and cover, surface litter was removed from a sub-set of quadrats, and then dried at 70°C for 48 hours. Dried litter was weighed and then ground to a fine particle size using a Thomas Wiley Mini-Mill (Thomas Scientific, Swedesboro, NJ) and subsamples (0.5 – 1.0 g) were sent to the University of Kentucky for analysis of percent C and N on a Flash Elemental Analyzer 1112 (Thermo Fischer Scientific Inc., Waltham, MA). From these data, we quantified changes in litter mass over time.

Statistical analysis

We used mixed effect models to assess treatment and elapsed years-since-fire (YSF) as fixed effects and year and pasture as random effects. Means were compared with a post hoc least squares test using the LSMEANS statement for pairwise comparisons of all combinations of treatment and YSF. Significance of ANOVA models was set at $P \leq 0.05$. All analyses were conducted in SAS 9.3 (SAS Institute 2011).

The burn patch within each pasture was considered the experimental unit (n = 36). Percent tall fescue cover, herbaceous litter cover, warm-season grass cover, woody cover, litter depth, litter mass, estimated litter biomass, and carbon: nitrogen (C:N) ratios were used as dependent variables. In some cases – particularly with the carbon and nitrogen content analyses – the response variable was log transformed to satisfy the homogeneity of variance assumption. In addition, Satterthwaite degrees of freedom were used to adjust for unequal variances. There was inconsistent application of the Twidwell et al. (2009) protocol for measuring litter biomass in 2012 as compared to 2013-2014 due to employee turnover. In 2012, the litter biomass estimation categories included a scale of 0-4, whereas the scale in 2013 and 2014 was 0-3. Therefore, we only analyzed the 2013 and 2014 litter biomass data.

Nutrient content dynamics were determined by multiplying the percent of carbon (or nitrogen) by the dry weight of each sample. Due to issues related to calibration of tare weights, some samples (24 out of 316) had a "negative" mass after drying the samples. To adjust for this problem, we deleted one point (an extreme outlier) that had a dry biomass of -10 g. All the other "low" biomass points were between -3.3 and 0 g. As per the recommendation of a statistical consultant (Lendie Follet, ISU), we ran an alternative analysis by adding an increment of 3.4 grams to all points. Thus, the means in this analysis

were all modified by a known increment, but the differences between years and years-since-fire could be more accurately accounted for.

We also used a mixed effect model with treatment and elapsed time-since-fire as fixed effects and year and pasture as random effects for the nutrient content dynamics. The response variable was log transformed to satisfy the homogeneity of variance assumption. In addition, Satterthwaite degrees of freedom were used to adjust for the unequal variances.

Finally, we applied a Pearson's correlation coefficient to test for possible linear relationships among plant functional group cover estimates and litter variables (SAS Institute 2011).

Results

Over the three-year study period, mean fescue cover did not decrease as a result of the fire and grazing treatments. There were differences in mean percent fescue cover among the treatments ($F = 10.09$; df = 2, 8.54; $P = 0.0056$), but these differences could be attributed to the fact that most of our burn-only (BO) control pastures had negligible fescue levels at the start of the study in addition to high variability in fescue cover in our graze-and-burn (GAB) and patch-burn-graze (PBG) pastures (Table 1). The GAB and PBG pastures had four to five times as much fescue cover as the BO pastures (Table 2). A means separation indicated that there was no difference in fescue cover between GAB and PBG pastures over the three years of the study ($t = 0.39$; df = 8.467, $P = 0.7068$). In fact, tall fescue cover increased slightly in the GAB treatment and fluctuated by year in the PBG treatment (Table 2). Years-since-fire also had no direct effect on mean fescue cover ($F = 0.64$; df = 2, 18.12; $P = 0.5363$ and see summary of years-since-fire in Table 5). When changes in mean fescue cover for PBG

pastures only, was tested there was no interaction between year and years-since-fire, but the main effect of year was significant (F = 15.64; df = 2, 330.1; P < 0.0001) (Table 3).

Across all three years there were no significant interactions between years-since-fire and treatment (F = 1.14; df = 4, 21.07; P = 0.364). In contrast, the main effect of years-since-fire was significant for litter cover (F = 33.72; df = 2, 17; P < 0.0001). The average litter cover in the burn year (0 YSF) was 28.8%, while the litter cover in 1 YSF was 48.6%, and 2 YSF was 58.5%. Within each treatment, the 0 YSF interval had a significantly lower percent litter cover than the one and two elapsed YSF (Figure 2).

A simple correlation between litter cover and litter depth was analyzed to corroborate our field estimates. Indeed, there was a positive relationship between litter cover and litter depth (r = 0.521, P < 0.0001). Not surprisingly, the litter depth results mirrored those of litter cover. The main effect of years-since-fire on litter depth was significant (F = 18.09; df = 2, 13.26; P = 0.0002). A mean separation showed that in all three treatments, litter depth in the most recently burned area (0 YSF) was significantly lower than depth in one and two elapsed YSF areas (Figure 3). In addition, in all three treatments, there was no difference in litter depth between the 1 YSF and 2 YSF. Importantly, these litter depth differences can all be observed each year within the PBG pastures, but the BO and GAB pastures have more homogeneous litter depths within each year.

Litter biomass was affected by the interaction between treatment and years-since-fire (F = 3.5; df 3, 10.59; P = 0.0548) and a mean separation analysis showed that litter biomass differed most between patches within the PBG treatment (Figure 4). Importantly, the biomass estimates for 2013-2014 and the litter depths were positively correlated (r = 0.751, P < 0.001).

No differences were detected among treatments in percent C in the litter ($F = 2.22$; df = 2, 25.91; $P = 0.1290$). Similarly, there was no effect of years-since-fire on percent C across treatments ($F = 0.18$; df = 2, 20.02; $P = 0.8373$). In addition, there was no effect of treatment on percent N ($F = 0.24$; df = 2, 8.445; $P = 0.7924$), nor was there an effect of years-since-fire on percent N across treatments ($F = 0.67$; df = 2, 21.01; $P = 0.5226$). No correlation was found between percent legume cover and percent N in the litter samples across treatments (r = -0.083; $P = 0.145$). Finally, there was no effect of treatment on C:N ratio ($F = 1.79$; df = 2, 8.773; $P = 0.2229$) nor years-since-fire on C:N ratio ($F = 0.65$; df = 2, 21.06; $P = 0.5328$). Averaged across all three treatments and years-since-fire, there seems to be a weak negative relationship between tall fescue cover and the C:N ratio (Table 4).

Carbon mass (g) was affected by an interaction of treatment and years-since-fire ($F = 3.23$; df = 4, 20.66; $P = 0.0330$) (Figure 5). However, overall nitrogen content was only affected by years-since-fire ($F = 15.06$; df = 2, 26.58; $P < 0.0001$). There was high variability in the N content analysis; but a separation of means showed all estimates were significantly different than 0 at $P < 0.10$.

Averaged across all treatments and years-since-fire there was a weakly positive, but significant, relationship between tall fescue cover and *litter cover*; however, tall fescue cover and *litter depth* showed a relatively stronger positive relationship (Table 4). We found no correlation of tall fescue cover with woody plant cover (r = -0.1086, $P = 0.0548$). In addition, there was no effect of treatment or years-since-fire on the percent cover of woody plants ($F = 0.21$; df 4, 10.67; $P = 0.93$). Average woody plant cover across all pastures in all three years of the study was 0.5%. For most tests, tall fescue had weak correlations with plant functional groups except warm season grasses and forb cover (Table 4). Warm season grass cover

differed significantly with an interaction of treatment and years-since-fire (F = 5.84; df = 4, 16.07; P = 0.0043), but this trend was driven by the high proportion of warm season grasses present in the BO control treatments; BO pastures had significantly higher warm season grass cover (averaging 44.3%) than either GAB (9.3%) or PGB, (14.4%). Percent cover of warm season grasses did not differ between the GAB and PBG treatments (t= -0.48; df = 8.72; P = 0.6455).

Discussion

The results of our study suggest that patch-burn grazing cannot significantly reduce tall fescue cover over time. We had hypothesized that cattle would consume more tall fescue in patch-burn graze pastures than in traditionally-managed pastures because they would select the burn patch and set back the competitive advantage of tall fescue in those pastures. Based on our litter depth results, we have evidence that cattle did indeed prefer to graze in the burn patch, and that likely increased their consumption of tall fescue in these locations. Despite the focal grazing, tall fescue cover did not decrease overall (Table 2). Indeed, tall fescue cover was not reduced as a result of any of our treatments, which is similar to other recent research indicating endophyte-infected tall fescue is tolerant to prescribed fire (Hall et al. 2014).

When analyzing differences in mean fescue cover for PBG pastures only, it is unclear why there was an increase in tall fescue cover between the years 2013 and 2014 (Table 3). Perhaps there were climatic differences between years, but these results do not support any of our hypotheses. One explanation for the higher fescue cover estimates in 2014 might be that half of the PBG pastures were switched to an early-intensive stocking system, so the cattle

were taken off the pastures earlier during the growing season than in previous years of the study. Since these cover estimate data were taken in the fall, it is plausible that the tall fescue generated more biomass in 2014 with the increased recovery time.

Increased time since fire was anticipated to influence litter quantities dramatically. We hypothesized that litter fuel loads would accumulate in unburned patches of the PBG treatment due to cattle focally grazing in burned areas. In contrast, we expected litter depth to remain relatively constant in GAB and BO treatments. In fact, years-since-fire was highly correlated with litter accumulation across all treatments, despite the large differences in fescue cover and warm season grasses (Figures 2-3). Overall, our litter accumulation results concur with those previously found in the patch-burn grazing literature (Churchwell, et al., 2008; Weir et al., 2013)

The percentage of litter nutrients (C and N) in each litter sample did not differ by treatment or time since fire. These results are expected for carbon because percent carbon is considered relatively constant across plant taxa (Schlesinger 1991). In contrast, we were somewhat surprised to find no differences in percent N, because N in plant tissue has been reported to differ among species, communities, age of plants, years-since-fire, and climate (An et al. 2005, Anderson et al. 2006). Similarly, because we expected N to be higher in litter from burned areas, we also expected to see differences in C:N ratios between the different fire return intervals (0, 1, and 2 years since fire). We did not find any such differences, but there was a weak negative correlation between C:N ratio and tall fescue cover (Table 4). This pattern might have been driven by the BO treatment, which had low fescue cover, but high C:N ratio. Warm season grasses, which dominated the BO pastures, tend to have higher C:N ratios than cool season grasses (Wedin and Tilman 1990), and cool season grasses such as

tall fescue were more abundant in GAB and PBG pastures. Within GAB and PBG, there was not a strong correlation between fescue cover and C:N ratio.

There was significantly higher litter cover and biomass two years-since-fire, so it is not surprising to find greater C and N content in the samples with increased years-since-fire (Figs. 5 & 6). Ideally, we would have collected soil nutrient measures and/or clipped biomass to validate our surface litter samples, however this was not possible. In addition, other metrics of litter quality could have been included such as lignin and or a lignin:nitrogen ratio (Bontti et al. 2009), which could influence rates of litter decomposition, and thus affect fuel loads for future burns.

As suggested previously, endophyte-infected tall fescue has the potential to be a particularly idiosyncratic challenge for restoration practitioners. Its unique qualities caused us to generate an alternative hypothesis; we indicated that tall fescue might resist fire due to its moist fuel-load during spring-time prescribed burns (McGranahan et al. 2013). Thus, we suggested tall fescue cover and litter cover would be positively related. We did not evaluate litter moisture in the spring nor "burn completeness," but we did find that sites with higher fescue cover had slightly deeper litter. Despite this moderately positive correlation, our results suggest that litter depth patterns may depend more on years-since-fire than percent tall fescue cover, regardless of the treatment. Additionally, we hypothesized that tall fescue presence might facilitate faster rates of woody plant invasion if prescribed fires were incomplete, but we did not find evidence of a relationship between tall fescue cover and woody plant cover (Table 4). Interestingly, both warm season grasses and forbs had negative relationships with tall fescue cover, which indicates that where tall fescue is abundant, warm season grasses and forb cover are significantly reduced. There is no evidence of causation in

this relationship, but the finding supports the idea that endophyte-infected tall fescue reduces plant community diversity (Clay and Holah 1999, Tunnell et al. 2004, Rudgers et al. 2005, 2007).

Several of these results are contrary to those of previous studies where plant invasions affected multiple aspects of the fuel-bed including litter depth and thus significantly altered fire regimes (D'Antonio and Vitousek 1992, Brooks et al. 2004). Even though many of our study sites had high levels of tall fescue cover (Table 1), it is possible that our litter composition was too heterogeneous to detect strong effects of tall fescue on overall litter dynamics within our system. It seems as though our fire and grazing disturbances maintained plant community diversity despite the presence of tall fescue.

Conclusion

Our research demonstrates that patch-burn grazing can be used to create discrete litter depths within a pasture. In patch-burn grazed pastures, litter was scarcest in the burn patches, which suggests that cattle preferred to graze there. However, the three grassland management techniques we employed did not alter average fescue cover in the pastures' plant community. In addition, we showed that years-since-fire altered litter dynamics more strongly than differences in percent cover of tall fescue. In terms of our fire return intervals, patterns of litter depth and litter cover were similar across all treatments. The major difference between treatments was that in patch-burn grazing, one patch per pasture is burned every year, while the other patches accumulated litter. Thus, the patch-burn grazing system provides a greater diversity of litter depths during any one year than in graze-and-burn and burn-only treatments. Such a mosaic of litter depths creates habitat heterogeneity and refugia that are

important for many insect and bird species (Vogel et al. 2007, Limb et al. 2009, Wolkovich 2010).

We suggested that tall fescue could potentially modify fire behavior because it is often alive and growing at the time of fire (McGranahan, 2012). Such moisture-rich fuel loads could cause incomplete burns, resulting in thicker litter layers developing in association with tall fescue presence. We found only a marginally significant relationship between litter depth and fescue cover. Given that many fires occur in the spring, additional research could provide valuable information regarding spring litter depth and moisture content.

Based on the results presented here, patch-burn grazing by itself may not eliminate or reduce tall fescue in these landscapes and herbicide application also may be necessary. However, our work indicates that patch-burn grazing can be a successful management tool in pastures that have high levels of tall fescue. Thus, there may still be a way to synergize cattle production and conservation goals in these systems.

Future research on the effects of tall fescue on litter dynamics ideally would include sites with high fescue cover in the burn-only category; however, such grasslands are uncommon in the Grand River Grasslands because most sites with high tall fescue cover are grazed. Nevertheless, including sites with high fescue dominance in a burn-only treatment would lend important additional insight into understanding methods to manage fescue with natural disturbances such as fire and/or grazing.

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Table 1. Mean percent tall fescue canopy cover (± standard error) in each pasture averaged across all patches over time. Treatments included burn-only (BO), graze-and-burn (GAB), and patch-burn-graze (PBG). See Figure 1 for site locations.

		Years Since Fire			
Treatment	**Pasture**[a]	**0**	**1**	**2**	**Average**
BO	KLT	1.8 ± 6.8	1.5 ± 3.3	4.3 ± 3.3	2.5 ± 9.9
	PAW	1.1 ± 3.3	0.6 ± 3.3	0.0 ± 6.8	0.8 ± 2.6
	RCH	45.8 ± 7.2	41.1 ± 3.3	NA[b]	41.9 ± 21.8
	RIN	0.0 ± 6.8	0.0 ± 3.3	0.1 ± 3.3	0.0 ± 0.3
GAB	GIL	82.2 ± 6.8	64.0 ± 3.3	76.2 ± 16.2	71.4 ± 19.3
	LTR	36.8 ± 6.8	35.8 ± 3.3	43.5 ± 27.9	39.4 ± 29.5
	PYW	32.1 ± 7.2	44.6 ± 22.5	70.2 ± 18.6	55.1 ± 24.9
	STE	34.4 ± 7.2	57.3 ± 22.2	69.3 ± 18.6	60.3 ± 22.9
PBG	KLN	54.4 ± 30.3	46.5 ± 27.8	47.8 ± 22.7	47.9 ± 25.7
	PYN	41.1 ± 14.3	42.4 ± 15.3	60.6 ± 18.1	50.4 ± 18.8
	PYS	72.1 ± 33.5	46.6 ± 19.7	60.5 ± 18.9	55.5 ± 22.6
	RIS	33.3 ± 34.4	34.2 ± 28.2	60.2 ± 23.7	45.8 ± 29.7

[a] KLT = Kellerton Tauke; PAW = Pawnee Prairie; RCH = Richardson; RIN = Ringgold North; GIL = Gilleland; LTR = Lee Trail; PYW = Pyland West; STE = Sterner; KLN = Kellerton North; PYN = Pyland North; PYS = Pyland South; RIS = Ringgold South (see

[b] RCH was removed from the study in 2014.

Table 2. Mean percent fescue canopy cover (± standard error) by year and treatment. (n = 4 for each treatment with the exception of 2014, when n = 3 for the Burn Only pastures.)

	Year			
Treatment	**2012**	**2013**	**2014**	**Average** [a]
Burn-only	11.8 ± 8.0	10.9 ± 7.4	11.0 ± 7.7	11.9 ± 8.6 a
Graze-and-burn	46.6 ± 8.0	50.4 ± 7.4	64.8 ± 7.4	54.9 ± 8.6 b
Patch-burn grazing	50.2 ± 7.8	42.4 ± 7.7	57.3 ± 7.2	49.3 ± 8.5 b

[a] Means within the Average column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's protected Least Significant Difference (LSD).

Table 3. Mean (± standard error) tall fescue canopy cover in patch-burn graze pastures by years-since-fire (YSF) and by year. Means containing the same letter are not different ($P \leq 0.05$) according to Fisher's protected Least Significant Difference (LSD).

YSF	Canopy cover (%)	Year	Canopy cover (%)
0	49.14 ± 3.22 a	2012	50.40 ± 4.27 ab
1	48.34 ± 3.17 a	2013	42.40 ± 2.51 b
2	52.65 ± 3.29 a	2014	57.33 ± 2.51 a

Table 4. Summary of correlations between tall fescue (TF) canopy cover, litter abundance and C:N ratio, and plant functional groups other than tall fescue across treatments.

Variables	Pearson's r	p
TF v. litter cover	0.1564	0.0056
TF v. litter depth	0.2141	0.0001
TF v. C:N ratio	-0.2248	< 0.0001
TF v. warm season grasses	-0.6046	< 0.0001
TF v. cool season grasses	-0.0004	0.9941
TF v. forbs	-0.3289	< 0.0001
TF v. legumes	0.0825	0.1455
TF v. woody cover	0.2141	0.0001

Table 5. Study pastures indicating the treatment and the burn schedule. The entire pasture was burned once every three years in burn-only and graze-and-burn treatments, whereas one out of three patches was burned every year on a rotation in the patch-burn-graze treatment.

			Years Since Fire		
Site Name	**Treatment**	**Patch**	**2012**	**2013**	**2014**
Kellerton Tauke (KLT)	Burn-only		0	1	2
Pawnee (PAW)	Burn-only		2	0	1
Ringgold North (RIN)	Burn-only		0	1	2
Richardson (RCH)	Burn-only		0	1	NA
Sterner (STE)	Graze-and-burn		0	1	2
Gilleland (GIL)	Graze-and-burn		0	1	2
Lee Trail (LTR)	Graze-and-burn		0	1	2
Pyland West (PYW)	Graze-and-burn		0	1	2
Kellerton North (KLN)	Patch-burn-graze	East	2	0	1
		Center	1	2	0
		West	0	1	2
Pyland North (PYN)	Patch-burn-graze	North	2	0	1
		South	0	1	2
		West	1	2	0
Pyland South (PYS)	Patch-burn-graze	North	0	1	2
		South	1	2	0
		West	2	0	1
Ringgold South (RIS)	Patch-burn-graze	North	1	2	0
		Center	0	1	2
		South	2	0	1

Note: Richardson was removed from the study in 2014 because there was a change in management inconsistent with our treatments.

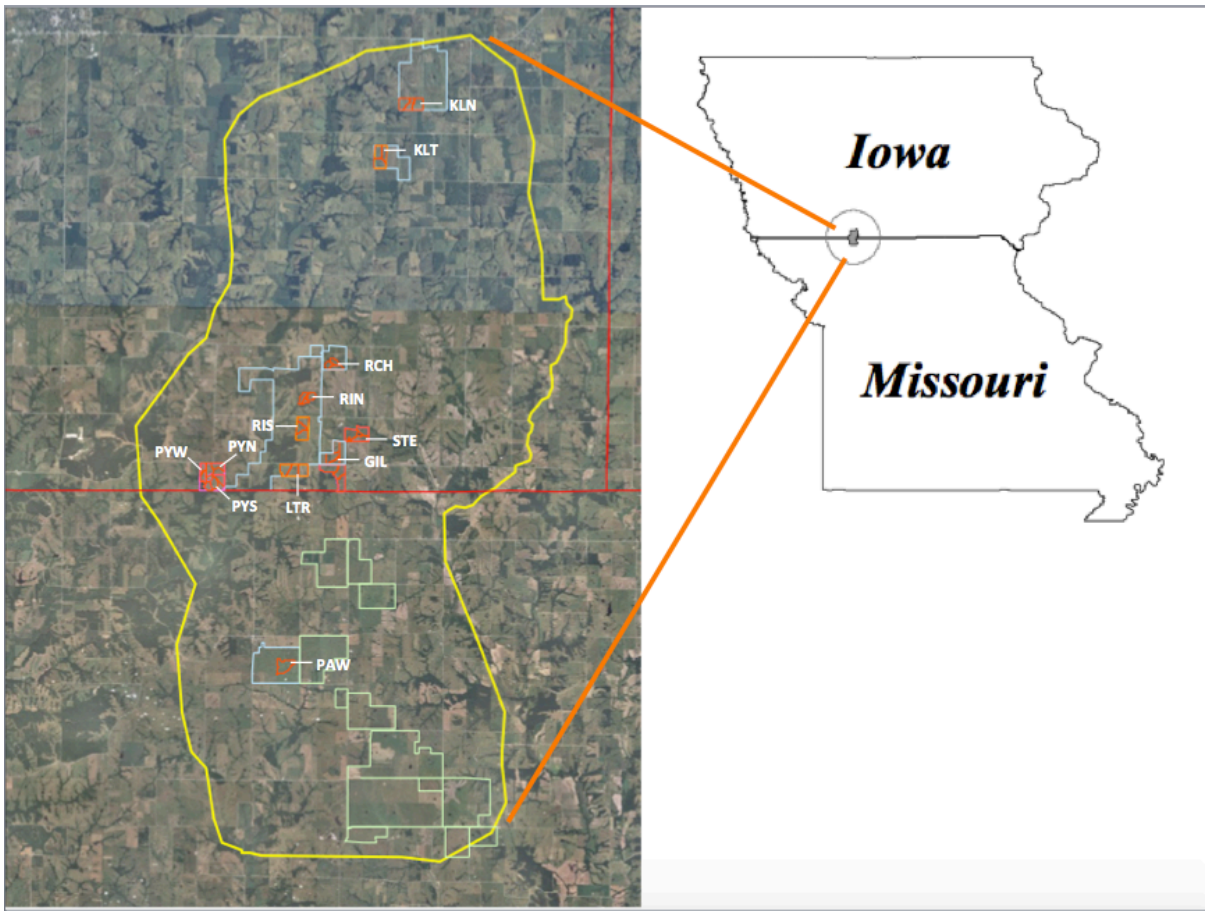

Figure 1. Map showing the Grand River Grasslands Conservation Priority Area (outlined in yellow). The red lines depict the county and state lines, while the 12 study sites are outlined in orange. State organizations manage lands outlined in blue, and The Nature Conservancy manages land outlined in green. Site names are as follows: KLN = Kellerton North; KLT = Kellerton Tauke; RCH = Richardson; RIN = Ringgold North; RIS = Ringgold South; PYW = Pyland West; PYN = Pyland North; PYS = Pyland South; LTR = Lee Trail; GIL = Gilleland; STE = Sterner; PAW = Pawnee Prairie.

Figure 2. Effect of treatment and years-since-fire on mean litter cover (± standard error), Grand River Grasslands, USA, 2012-2014 (0 YSF = 0 years-since-fire, 1 YSF = 1 year since fire, 2 YSF = 2 years since fire). Significance of ANOVA models was set at $P \leq 0.05$.

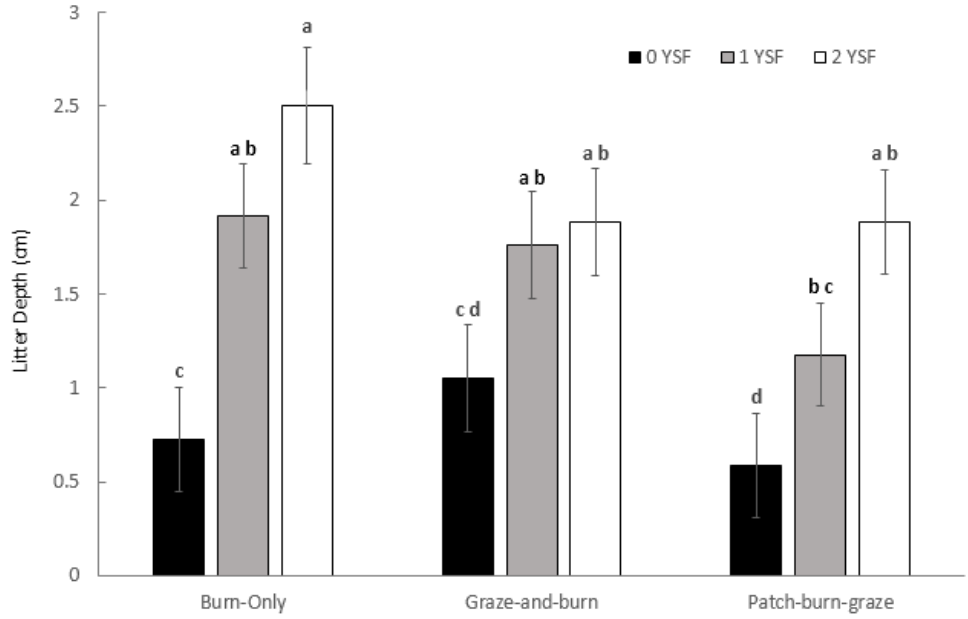

Figure 3. Effect of treatment and years-since-fire on mean litter depth (± standard error), Grand River Grasslands, USA, 2012-2014 (0 YSF = 0 years-since-fire, 1 YSF = 1 year-since fire, 2 YSF = 2 years-since-fire). Significance of ANOVA models was set at $P \leq 0.05$.

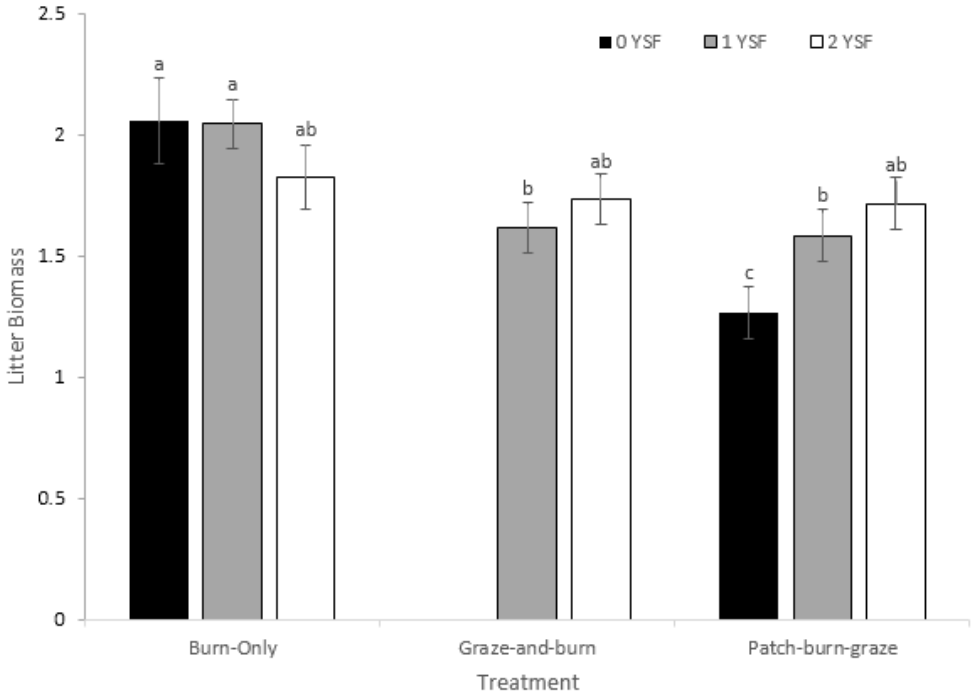

Figure 4. Estimated litter biomass within each treatment using Twidwell (2009) categories 0, 1, 2, 3 with 0 representing the lowest litter mass and 3 the highest litter mass. Data are from 2013 and 2014 only, and thus n = 1 for Burn-Only 0 YSF (Pawnee Prairie). Error bars represent standard error of the mean.

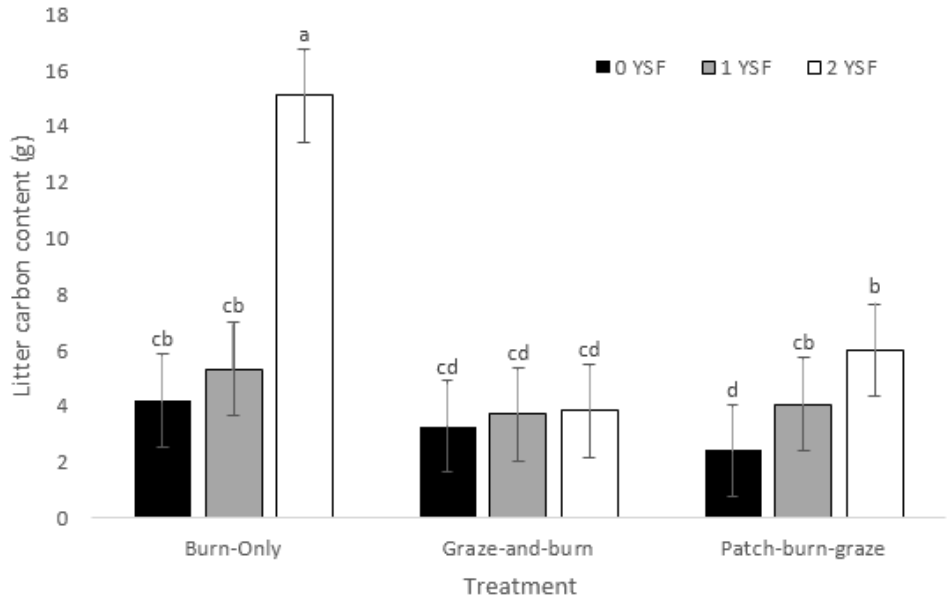

Figure 5. Mean (± standard error) carbon mass (g) in litter samples from three different treatments and fire return intervals: 0 YSF = 0 years since fire, 1 YSF = 1 year since fire, 2 YSF = 2 years-since-fire. Significance of ANOVA models was set at $P \leq 0.05$.

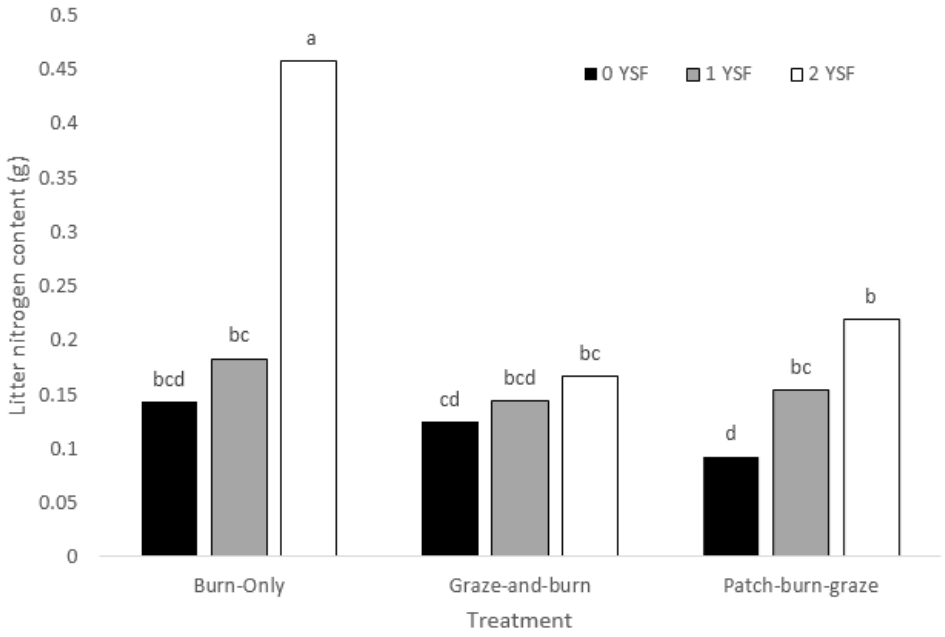

Figure 6. Mean nitrogen mass (g) in litter samples from three different treatments and fire return intervals: 0 YSF = 0 years-since-fire, 1 YSF = 1 year-since-fire, 2 YSF = 2-years-since fire. Significance of ANOVA models was set at $P \leq 0.10$. Error bars were omitted due to high levels of variation.

CHAPTER 3. EFFECTS OF TALL FESCUE AND ITS FUNGAL ENDOPHYTE ON THE DEVEOPMENT AND SURVIVAL OF TAWNY-EDGED SKIPPERS (LEPIDOPTERA: HESPERIIDAE)

A paper accepted for publication in *Environmental Entomology*

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Abstract

Invasive, exotic grasses are increasing in tallgrass prairie and their dominance may be contributing to the decline of grassland butterflies through alterations in forage quality. Tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort), an exotic grass covering millions of acres in the United States, can host a fungal endophyte, *Epichloë coenophiala* (Morgan-Jones & Gams). Alkaloids produced by the endophyte are known to be toxic to some foliar-feeding pest insects. Endophyte-infected tall fescue is commonly planted in hayfields, pastures, lawns, and is invading natural areas, but effects of the endophyte on non-pest insects such as butterflies are relatively unknown. Our objective was to investigate the role that tall fescue and its endophyte might play in the decline of grass skippers (Hesperiidae). We examined growth and survival parameters of tawny-edged skippers (*Polites themistocles* (Latreille)) that were reared on endophyte-infected tall fescue (E+), endophyte-free tall fescue (E-), and Kentucky bluegrass (KBG). KBG was included as a comparison because it is a cool season grass known to be palatable to *P. themistocles* larvae. Interestingly, results showed that the endophyte did not affect growth and survival of larvae compared to uninfected tall fescue, even though significant amounts of loline alkaloids (average 740 ppm) were measured in endophyte-infected plant material. Larvae feeding on KBG grew faster with greater survival

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rates than larvae on both tall fescue treatments. These results confirm that tall fescue invasion and dominance may be deteriorating the quality of grassland habitats for native pollinators; however, this effect does not appear to be linked to endophyte infection.

Key words

Schedonorus arundinaceus (Schreb.), Epichloë coenophiala, loline alkaloid, *Poa pratensis, Polites themistocles*

Introduction

Many of the world's grassland ecosystems are disappearing and undergoing major compositional changes (Ceballos et al. 2010, Veldman and Putz 2011, Parr et al. 2014). North American tallgrass prairie ecosystems have declined in area by 99.9% since the mid-1800s, largely due to the conversion of land for agricultural uses (Samson and Knopf 1994). Much of the grassland habitat remaining globally exists as novel mixtures of exotic and native species in pastures, hayfields, and rangelands (Herkert et al. 1995, Hobbs et al. 2009, Veldman et al. 2015). These changing plant communities affect the distribution and abundance of insect communities associated with grasslands (Jonas et al. 2002). An extreme example exists in New Zealand where the pastures have been largely reduced to one introduced grass species and one species of clover, and the control of insect pests has become increasingly complex due to the simplified landscape (Goldson et al. 2014). In the United States, exotic and pervasive grasses such as tall fescue, Kentucky bluegrass, and smooth brome (*Bromus inermis*) can account for at least half of the vegetation in some grassland systems (Grant et al. 2009), and these species are likely modifying the activity and viability of local grassland wildlife, including pollinators. For example, tall fescue (*Schedonorus*

arundinaceus (Schreb. Dumort), formerly *Lolium arundinaceum* (Schreb.)), a Eurasian cool-season grass, covers millions of acres in North America (Fribourg et al. 2001) and is now the most abundant cool-season grass in the eastern United States (Ball et al. 1993, Roberts and Andrae 2004, Rudgers and Clay 2007). Moreover, an estimated 75-80% of tall fescue in North America is infected with the fungal endophyte *Epichloë coenophiala* (Morgan-Jones & Gams) (formerly *Neotyphodium coenophialum*) (Leuchtmann et al. 2014), which produces alkaloids that cause toxicosis in cattle and are detrimental to insect herbivores (Ball et al. 1993, Roberts and Andrae 2004, Rudgers and Clay, 2007).

There are four major classes of alkaloids known to be produced by *Epichloë spp.,* and all of them have been shown to deter at least some types of invertebrates (Cheplick and Faeth 2009). Peramine and loline alkaloids are the most frequently implicated in invertebrate resistance (Clay 1989), though they may operate in different ways against insect herbivores, (e.g.such as deterring feeding or decreasing growth and reproduction) (Hoveland 1993, Blankenship et al. 2001). In particular, lolines have been associated with lower insect biomass, increased mortality, and longer developmental times (Wilkinson et al. 2000, Jensen et al. 2009). However, results from some studies show that alkaloid effects on invertebrates are somewhat equivocal (Saikkonen et al. 2006), and effects seem to vary across species of herbivores (Crawford et al. 2010). Most negative alkaloid effects come from studies that used generalist pest insects (Siegel et al. 1990, Wilkinson et al. 2000, Braman et al. 2002, Roberts, West, and Spiers 2005, Ball et al. 2006, Baldauf, Mace, and Richmond 2011, Keathley and Potter 2012). In spite of extensive efforts to describe the role of endophytes in modifying insect herbivory, relatively little is know about the ways in which endophyte-infected tall fescue might be impacting non-pest insects in more natural grassland communities.

In the Midwestern United States, there have been major declines in many prairie-specialist butterflies, as well as many other important native pollinators (Swengel et al. 2011, Swengel & Swengel 2015). Generally, the causes of these declines are attributed to habitat loss and fragmentation and to the extensive use of herbicides and pesticides in agriculture (Wright and Wimberly 2013, Vanbergen and the Insect Pollinators Initiative 2013). However, one of the more puzzling issues is the conundrum of skipper butterfly losses despite the apparent plethora of grassland habitat in some regions. Given that many skippers use grasses as host plants, the driving factor of observed declines is clearly not attributable to habitat loss. Furthermore, if the sites receive limited insecticide use, as is common in many US grasslands (United States Department of Agriculture 2003), then there must be additional factors contributing to pollinator declines. Grass skippers from the subfamily Hesperiinae are grass-eating generalists in their larval stages, yet they are failing to thrive in many grassland environments. One possible reason for their decline is that overall forage diversity and quality is changing due to increased exotic grass cover. Another potential threat may be that of fungal endophyte toxins produced within the plant tissue of common invasive exotic pasture grasses such as tall fescue. Given that several grass skippers are being proposed for listing as endangered or threatened species (Iowa Natural Resource Commission 2009, Fish and Wildlife Service 2014), it is imperative to investigate the role that altered forage quality, particularly alkaloids associated with endophyte-infected tall fescue, might be playing on these prairie specialists.

The objective of this research was to to compare the effects of endophyte-infected (E+) and endophyte-free (E-) tall fescue versus a common, co-occurring cool season grass, Kentucky bluegrass (*Poa pratensis*) (KGB) on the growth and development of Tawny-edged

skippers (*Polites themistocles*). While *Panicum* species have been reported as host plants for Tawny-edged skippers in some parts of their range, their grass preferences in Iowa are presently unknown (Schlicht et al. 2007). Several sources suggest *P. pratensis* as a potential host plant for this species (Scott 1986, James and Nunnallee 2011, Opler et al. 2012). Tawny-edged skippers are common residents of tallgrass prairies, but they also are frequently seen in old fields, city parks and urban lawns. We used this butterfly because it is a native species, but much more numerous than some of the more threatened species. Larvae of *P. themistocles* are known to feed on a wide variety of grasses (Scott 1986, James and Nunnallee 2011) and they are, therefore, likely affected by increasing exotic grass dominance. We hypothesized that larvae grown on E+ tall fescue would have reduced survival and reduced weight gain compared to larvae grown on E- tall fescue and KBG because of the negative effects of loline alkaloids. We also hypothesized that there would be minimal response differences between E- tall fescue and KBG. Finally, we expected that these laboratory based results would provide important insights about the impacts of endophyte-infected tall fescue on the life history of prairie-dependent butterflies.

Methods

Plant material

Three types of grass were used as treatments in this study: endophyte-infected tall fescue 'KY-31' (*Schedonorus arundinaceus* Schreb.) (E+), endophyte-free tall fescue 'KY-31 EF' (E-), and Kentucky bluegrass (*Poa pratensis*) (KBG). We chose tall fescue 'KY-31' because it is the most widespread cultivar grown in the United States (Ball et al. 1993), and it has invaded many natural areas within the tallgrass prairie ecoregion (McGranahan 2012).

KBG was selected because it often co-occurs as an invasive, exotic grass with tall fescue in tallgrass prairies (Cully et al. 2003), and previous efforts rearing tawny-edged skippers (*Polites themistocles*) were successful on this grass (James and Nunnallee 2011). Thus, KBG served as a baseline (a known palatable species) to which we could compare to E+ and E- tall fescue. Grass seeds were sourced from Dr. Tim Phillips, a grass breeder in the Department of Plant and Soil Sciences at the University of Kentucky.

Grass plugs were grown in 72-cell trays in a greenhouse. Tall fescue was seeded at a rate of 10-15 seeds per cell, whereas KBG was seeded at a higher rate (15-20 seeds per cell) due to smaller seed size. Four- to eight-week-old grass plugs were used as forage for the developing larvae.

The grass plugs were replaced for each larva on a routine basis in order to resupply forage and to avoid mold and frass build-up in each tube. The fescue grasses tended to grow more biomass in a shorter amount of time than KBG. Consequently, KBG grass plugs were smaller and needed to be replaced more frequently than tall fescue. On average, larvae feeding on KBG had their plugs replaced 6.2 times, whereas larvae feeding on the two fescue types had their plugs replaced an average of 4.6 times before they pupated. In addition, the KBG grasses were on average 4.6 weeks old, whereas the fescues were usually on average 5.0 weeks old when they were replaced.

Alkaloid, carbon, and nitrogen analyses.

Aboveground samples of all three grass types at 4, 5, 6, 7, and 8 weeks of age were sent to the University of Kentucky for alkaloid, and carbon and nitrogen analysis. Loline and ergot alkaloid analyses were conducted using gas chromatography-mass spectrometery (GC-MS) and high performance liquid chromatography (HPLC) techniques, the same as those

reported in McCulley et al. (2014). We expected alkaloid levels to differ as the plant matured (Hardy et al. 1986). The limit of detection for ergot alkaloids was 0.02 ppm and 10 ppm for loline alkaloids.

Percent C and N in the grass samples were assessed using a Flash Elemental Analyzer 1112 (Thermo Fischer Scientific, Inc. Waltham, MA). From these data we were able to calculate a C:N ratio in order to obtain a simple measure of forage quality in the larval diets.

Field collection of butterflies.

Female tawny-edged skippers can lay up to 300 eggs per year (Scott 1986). To ensure a reasonable sample size for this experiment, two assumptions were made: 1) Wild-caught butterflies already will have already laid some eggs in the field, and thus fewer than half of their total eggs would be available for the experiment. Therefore, we conservatively estimated that female butterflies in captivity would lay an average of 50 eggs per individual. 2) Regardless of treatment effect, developing larvae would have high mortality rates (50%) at each life stage in the rearing process. Given the expected losses throughout the experiment, we estimated that a minimum of 25 female butterflies should be collected from the field.

Butterflies were captured 11-20 June 2014 in the Grand River Grasslands of Ringgold County, Iowa. The Grand River Grasslands region is over 28,000 ha in size and composed primarily of perennial grassland. Small parts of the region have remnant tallgrass prairies, but the rest is highly invaded with tall fescue and other exotic species, including Kentucky bluegrass. Twenty-seven female tawny-edged skippers were captured with butterfly nets. In most cases, butterflies were found in restored prairies while nectaring on red clover (*Trifolium pratense*) or pale purple coneflower (*Echinacea pallida*). All individuals were transported back to the lab in cool, dark conditions to minimize harm during transport.

Insect rearing.

Subsequent stages of the experiment were conducted in a controlled environmental chamber (Carroll Coolers Inc., model N016898, Carroll, IA) at 25°C and 60% relative humidity, with a photoperiod of 16 hours of light and 8 hours of darkness.

After field collection, 5-8 butterflies were placed into each oviposition chamber. Oviposition chambers were constructed using two paper cups (one stacked within the other), with mesh netting fastened to the top. Each chamber contained a live KBG plug. Artificial nectar (1 part honey to 9 parts water) was applied to cotton swabs that extended into the chamber through holes in the side of the cup. Eggs were laid on the grass, as well as virtually all surfaces of the oviposition chamber. Twenty-seven females laid a total of 1,095 eggs.

In order to manage environmental conditions and account for each individual, eggs were transferred to egg chambers to hatch. To construct egg chambers, 8 oz. plastic containers were disinfected with 75% ethanol, and lined with a damp paper towel for humidity regulation (Fig. 1). The eggs were monitored daily, and hatching larvae were transferred to E+, E-, or KBG grass treatments. The first larva that hatched was assigned to E+, the second larva was assigned to E-, and the third larva was assigned to KBG. All subsequent larvae were assigned to treatments in the same order to assure equal sample size across treatments. Of the 1,095 eggs, only 617 produced larvae, most of which emerged after 7 to 9 days.

Developing larvae were reared in T12 clear polycarbonate tubes used for shatter guards on fluorescent light bulbs (1000 Bulbs, Garland, TX). Tubes were cut to 25 cm lengths, grass plugs inserted into the bottom of each tube, a single larva transferred onto a blade of grass within the tube and the tube capped with a fine screen to contain larvae Grass

plugs were inserted into the bottom of each tube and a single larva was transferred onto a blade of grass within the tube (Fig 1). Plugs were set onto paper towel-lined trays and watered from the bottom daily so that the plugs were damp but not saturated. All tubes were labeled with unique identification numbers so that individual larvae could be measured repeatedly over time.

The weights of individual larvae were measured every 5 days. Beyond these routine measurements, handling of larvae was kept to a minimum and was always done with disinfected equipment. Grass plugs were removed and replaced when larvae had consumed them, or when grasses became contaminated with mold or frass.

Once pupated, individuals were removed from the tubes, measured for mass and length, and affixed to twine with a small dab of silicone adhesive on the pointed tip of the chrysalis. They were then separated and placed in individually labeled paper-cup chambers where they could eclose yet remain contained (Fig. 1). Upon eclosion, adults were frozen for future analyses.

Data analysis.

All analyses were conducted in SAS 9.3 (SAS Institute 2011, Cary, NC). A two-way analysis of variance (ANOVA) (PROC GLIMMIX) was used to test for differences in C:N ratios and alkaloid concentration with grass treatment and age of grass samples as main effects.

A logistic, nonlinear mixed model was used to analyze the larval growth data. The model is as follows:

$$y_{ij} = \frac{b_1 + u_{1i}}{1 + exp[-(Time_{ij} - b_2)/b_3]} + e_{ij}$$

where $Time_{ij}$ is the corresponding time; b_1, b_2, and b_3, are the fixed effects parameters; u_{1i} is the random-effect parameter assumed to be independent and identically distributed (idd) N(0, $\sigma^2 u$), e_{ij} is the residual error assumed to be idd (0, $\sigma^2 e$) and independent of the u_{1i}. The growth asymptote for each treatment is defined by b_1, b_2 is the day(s) at which the maximum growth inflation occurred, and b_3 is the rate of growth. Y_{ij} represents the response of the j^{th} observation under the i^{th} treatment. This model allows the maximum growth achieved (b_1) and the rate at which each individual achieved it (b_3) to vary based on the treatment. Model parameters were compared across treatments using contrasts.

Larval survival data were analyzed using the nonparametric Kaplan Meier model (PROC LIFETEST), which calculates the probability of survival for each treatment group and at each time point. A log rank test was used to compare the probability of survival to adulthood between the three treatments in the long-term. A Wilcoxon test was used to detect differences in survival between groups in the short-term. Individuals were considered right-censored if they developed normally and eclosed as adults; these individuals generally developed faster and "withdrew" from the study early. All other individuals died prematurely as larvae or pupae, presumably due to treatment effects.

Analysis of variance was used to compare pupal responses in days to pupation, number of days as a pupa, pupal length, and pupal weight. All data were checked for normality, and a Poisson distribution was fit for age at pupation because the data were right-skewed. Means were compared using Fisher's least significant difference test, with significance set at $P = 0.05$.

Results

Grass forage quality.

The carbon to nitrogen (C:N) ratio was different across grass treatments ($F = 63.04$; df = 2, 49; $P < 0.001$). There was no difference between endophyte-infected and endophyte-free tall fescue, but on average, the C:N ratio for the two fescue treatments was 3.3 units higher than for Kentucky bluegrass. Low C:N ratios imply more nitrogen available per unit of carbon for feeding larvae. There were also differences in C:N ratios among different aged cohorts of grass ($F = 54.35$; df = 2, 49; $P < 0.001$). Averaged across all grass treatments, C:N tended to increase with age from a ratio of 7.4 for 4-week old grass samples to 13.2 for 8-week old samples. There was no interaction between grass treatment and age (weeks) ($F = 1.29$; df = 8, 49; $P = 0.27$).

Grass alkaloid profiles.

Alkaloid profiles consisted of various ergot and loline alkaloids (Table 1). As expected, E- tall fescue and KBG had barely detectable levels of both measured types of alkaloids; total loline levels for E- tall fescue were on average 13 ppm, and KBG had an average of 11 ppm. In contrast, total loline levels for E+ tall fescue were on average 740 ppm. Consequently, we only analyzed differences in alkaloid concentration across different age classes in E+ plant material (Table 1). E+ tall fescue had high levels of loline alkaloids, but unexpectedly low levels of ergot alkaloids. Interestingly, the loline alkaloid levels were consistently higher in younger E+ samples than in older samples. Total loline levels in four-week-old E+ material were seven times higher than in eight-week-old E+ material.

Skipper responses.

Larval growth asymptotes did not differ among the three grass treatments for weight gain (Table 2). The average maximum body weight across treatments was 0.26 (± 0.01 SE) grams with the rate of weight gain differing significantly among all three treatments (Fig. 2).

Overall, the long-term probability of larval survival did not differ among treatments according to the log rank survival analysis ($P = 0.0918$) (Fig. 3). Larvae that were fed on E+ and E- tall fescue had nearly identical long-term probabilities of survival, while those that fed on KBG had a higher survival probability during the first 40 days of development. A Wilcoxon test showed that there were significant differences in the short-term survival probabilities across treatments ($P = 0.0022$). Only 36.6% of the individuals reared on KBG died prematurely (n = 194), compared with 53.2% for E- tall fescue (n = 205) and 53.4% for E+ tall fescue (n = 206).

Similar to larval growth and survival responses, pupae reared on KBG developed faster and had greater overall biomass than individuals reared on the two fescue treatments (Table 3). Individuals reared on KBG pupated an average of 10 days earlier than those reared on tall fescue. There was no difference between E+ and E- treatments in days to pupation or days as a pupa, though differences were found in pupal length and weight. E+ pupae averaged 0.4 mm longer and 9.9 mg heavier than E- pupae (Table 3). Pupae from skippers fed KBG were the largest, averaging 0.5 and 0.9 mm longer, and 13.6 and 23.5 mg heavier, than E+ and E-, respectively (Table 3).

Discussion

Kentucky bluegrass and tall fescue have become dominant components of native U.S. tallgrass prairie ecosystems (Spyreas et al. 2001, Cully et al. 2003, Tunnell et al. 2004) and may negatively impact food webs, grassland community dynamics, and ecosystem processes (Rudgers and Clay 2007, McGranahan et al. 2012, 2015). Our study is the first to examine the effects of these exotic grasses on native butterflies by exploring the role of fungal endophyte infection in tall fescue. We found that tawny-edged skippers had enhanced growth, development, and survival on KBG versus tall fescue and that endophyte effects were limited.

Because numerous studies have described the anti-herbivore effects of endophyte-infected tall fescue on pest insects of turf and forage grasses (Ball et al. 2006, Cheplick and Faeth 2009), we hypothesized that alkaloid-containing (E+) tall fescue would be more deleterious to the development and survival of tawny-edged skippers when compared to alkaloid-free (E-) tall fescue and KBG. Contrary to our predictions, presence of the tall fescue endophyte did not affect growth or survival of larvae compared to E- tall fescue (Fig. 2 and 3). This result is surprising given that loline alkaloid levels were on average 57 times higher in E+ than E- tall fescue samples. Our results contrast with much of the literature that establishes *Epichloë*-associated loline alkaloids as toxic to invertebrate herbivores (Siegel et al. 1990, Bush et al. 1993, Wilkinson et al. 2000). However, there is some controversy about the role of endophyte-produced alkaloids in insect deterrence, as many people have also reported variability in insect responses (Saikkonen et al. 1998, Cheplick and Faeth 2009). Our findings illustrate that endophyte-produced loline alkaloids, at the levels seen in our study, are apparently not toxic to tawny-edged skippers.

Several researchers have used lepidopteran pest species (e.g. *Spodoptera frugiperda*) to quantify the effects of fungal endophytes on invertebrate herbivores. Our results both support and contradict this literature. For example, Boning and Bultman (1996) found that *S. frugiperda* larvae weighed less and took longer to develop after feeding on E+ tall fescue vs. E- plant material. In contrast, Bultman and Bell (2003) found the surprising result that *S. frugiperda* larvae had enhanced performance on E+ versus E- tall fescue. Our findings run counter to both of these results; for most parameters, skipper larvae performed similarly on E+ versus E- tall fescue, but the larvae on E+ tall fescue had a slightly slower growth rate than larvae reared on E- tall fescue (Table 2). Despite having dissimilar larval responses compared to those reported by others, our pupal responses concur with past research. On average, our E+ pupae were larger than E- pupae (Table 2), which is comparable to results reported by Boning and Bultman (2006). Because pupal mass in lepidoptera has been strongly associated with fitness (Campbell 1962, Hough and Pimentel 1978, Loewy et al. 2013), the endophyte might be conferring a benefit to these herbivores, but additional research is necessary to further explore this possibility.

One explanation for our unique results could be that native, grass feeding invertebrates such as the tawny-edged skipper, may have evolved in an environment with alkaloid-producing fungal endophytes (Tibbets and Faeth 1999). Research has shown that endophyte-infection is seen in ~2000 grass host species, especially among cool-season, C_3 grasses (Leuchtmann 1992, Clay and Schardl 2002, Rudgers et al. 2009, Crawford et al. 2010). Very few researchers have tested herbivore resistance in native endophyte-grass associations, but there is evidence that endophytic fungi may confer anti-herbivore benefits

to native grasses (Afkhami and Rudgers 2009, Crawford et al. 2010). This may explain why skipper larvae performed better than expected on E+ tall fescue.

We also assumed that larvae reared on E- tall fescue and KBG would have similar growth and survival rates because, without alkaloids, the forage quality of these two grass treatments would be comparable from the skipper perspective. The results indicated that KBG provided higher forage quality for tawny-edged skipper larvae compared to both E+ and E- tall fescue. KGB material had a lower C:N ratio and supported greater survival rates in the first 40 days of development (Fig. 3) than the fescue material. Larvae in the KBG treatment grew faster, larger, and with less variability (Fig. 3). Pupae reared on KBG also were distinctly heavier than pupae reared on either fescue. The difference in C:N ratios might be explained by the fact that our KBG grass plugs grew less biomass over time than the fescue plugs. Consequently, the KBG plugs required more frequent replacements, and the plugs were younger, and possibly more tender when they were being consumed.

One possible biological implication of the differences that we observed between KBG and both fescue treatments may have to do with age to pupation and rate of larval growth. Short development times and high growth rates are generally considered beneficial for insects (Nylin and Gotthard 1998) because prolonged development results in more exposure to predators and parasites, which can increase juvenile mortality (Sibly and Calow 1986). We suspect that a shorter age to pupation on KBG would have a positive effect on survival in a field setting, but additional research is needed to confirm this possible advantage.

The life history of many grass skippers is poorly known (Bouseman et al. 2006). For example, it is unclear how skippers and other grassland-dependent butterflies interact with exotic grasses, such as KBG and tall fescue, in the field. To our knowledge, no one has

observed tall fescue as a host plant for grass-eating butterfly species in North America, and only a few butterfly species have been observed to oviposit on KBG in a natural setting (Graves and Shapiro 2003, Beyer and Schultz 2010). For many skipper species laboratory studies have demonstrated larval preferences among various grass species, but very few observations have been made of oviposition events and larval feeding in the field (James and Nunnallee 2011). In our study it may have been more informative to include a broader suite of grass treatments to quantify developmental responses on co-occurring native grasses such as little bluestem (*Schizachyrium scoparium*) – a known host plant for some threatened grass skippers – and crabgrass (*Dichanthelium clandestinum*) – a reported host plant for *P. themistocles* (Scott 1986). Many of the threatened and endangered skippers such as the Dakota skipper (*Hesperia dacotae*) and Poweshiek skipperling (*Oarisma poweshiek*) have been reported to feed on warm-season grasses, which can also host fungal endophyte symbionts (Ghimire et al. 2011). Future research could compare our results with those of other grass-eating butterflies.

Our results illustrate that exotic grasses vary in their effects on insect forage quality. In the case of the tawny-edged skipper, tall fescue, regardless of endophyte infection status, provided lower quality forage than KBG. Tall fescue may thus effectively contribute to habitat loss by reducing forage quality, as well as decreasing overall plant community richness and diversity and altering grassland successional dynamics (Clay and Holah 1999, Rudgers and Clay 2007). Our results do not provide direct evidence that endophyte-produced loline alkaloids contribute to grass skipper declines. In fact, skipper pupae were larger on E+ vs. E- tall fescue, suggesting a possible benefit of alkaloid consumption. Further research exploring the fitness consequences of alkaloid consumption for this butterfly, as well as

additional native lepidopteran pollinators, seems warranted. In addition, research exploring other possible causes of grass skipper decline, including the effects of pesticide drift, habitat modifications, as well as oviposition habitat selection and host plant preference in the field would be useful. Our research has important implications for wildlife biologists, turf superintendents, and grassland restoration managers who manage areas with exotic invasive cool-season grasses. It highlights the fact that beyond the total number of acres restored, grassland composition, and particularly exotic species composition, is an important factor governing insect forage quality and should be considered in efforts that seek to improve habitat for pollinators and other native insects.

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Table 1. Alkaloid concentrations of endophyte-infected tall fescue above-ground plant material at different ages (weeks). Units are in parts per million (ppm).

	Alkaloids						
	Ergovalines[b]			Lolines[c]			
Age	EV [a]	EVI	Total erg	NANL	NFL	NAL	Total lol
4	0.026	0.016	0.042	86.4a	1308.2a	510.2a	1904.8a
5	0.106	0.058	0.164	32.2b	428.0b	225.8b	686.2b
6	0.026	0.002	0.026	23.2bc	315.8bc	189.2bc	528.2bc
7	0.026	0.000	0.028	16.2c	205.5c	127.4cd	348.8cd
8	0.016	0.000	0.010	9.8c	136.0c	87.6d	233.2d
	NS	NS	NS	***	***	***	***

[a] Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's protected LSD. *$P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant; values in columns were tested for significance.
[b] EV = ergovaline; EVI = ergovalinine; total erg = ergovaline + ergovalinine.
[c] NANL = *N*-acetyl norloline; NFL = *N*-formyl loline; NAL = *N*-acetyl loline; total lol = NANL + NFL + NAL

Table 2. Growth means and standard errors in terms of weight gain (g) from non-linear mixed effects model of Tawny-edged skipper larvae grown on three different grass diets: Kentucky bluegrass (KBG), endophyte-infected tall fescue (E+), and endophyte-free tall fescue (E-). P-values are presented for the contrasts of model parameters, with significant contrasts bolded.

Treatment	Growth asymptote (b_1)	Day of max growth (b_2)	Rate of growth (b_3)
E+	0.265 ± 0.009	29.1 ± 0.30	5.03 ± 0.16
E-	0.263 ± 0.009	29.7 ± 0.36	5.59 ± 0.19
KBG	0.266 ± 0.008	22.6 ± 0.19	3.75 ± 0.11
Contrasts			
E+ v. E-	0.8481	0.1912	**0.0209**
KBG v. E+	0.9716	**< 0.0001**	**< 0.0001**
KBG v. E-	0.8115	**< 0.0001**	**< 0.0001**

Table 3. Average pupal measurements and standard errors of individuals reared on three different grass diets: endophyte-infected tall fescue (E+), endophyte-free tall fescue (E-) or Kentucky bluegrass (KBG). Means containing the same letter are not significantly different from one another at alpha = 0.05. P-values are shown with significant differences bolded.

Treatment	*n*	Days to pupation	Days as pupa	Pupal length (mm)	Pupal weight (mg)
E+	105	43.0 ± 0.6a	8.8 ± 0.1	17.3 ± 0.09a	171.2 ± 2.3a
E-	112	43.8 ± 0.6a	8.9 ± 0.1	16.9 ± 0.09b	161.3 ± 2.2b
KBG	140	34.4 ± 0.5b	9.0 ± 0.1	17.8 ± 0.08c	184.8 ± 2.0c
		< 0.0001	0.7216	**< 0.0001**	**< 0.0001**

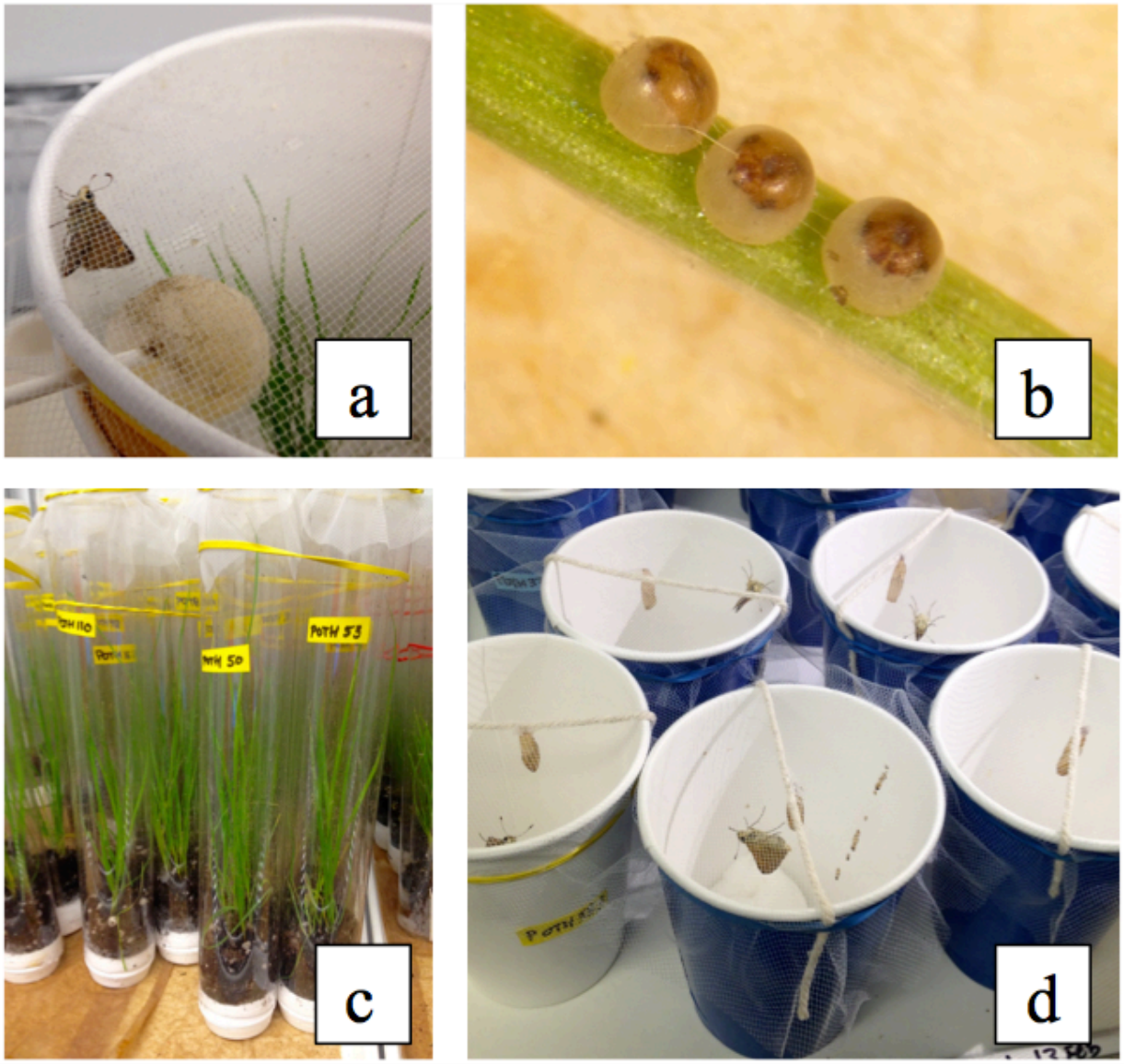

Figure 1. (a) Oviposition chamber with female skippers, a grass host plant (*Poa pratensis*), and a nectar source. Mesh netting was banded to the top of each cup. (b) Incubation chamber where eggs were transferred to eclose. Most eggs were removed from the oviposition chamber with a sterilized paint brush, but some eggs resisted removal, so we cut around the egg and left a small piece of grass attached to the egg. The incubation chamber was sterilized and contained a damp paper towel for humidity regulation. (c) Polycarbonate tubes with grass treatments and independent larvae. Each tube was capped with a fine mesh netting. (d) Pupation chambers made from paper cups, mesh netting, a dampened cotton ball for localized humidity, and individual pupae affixed to twine with silicone adhesive.

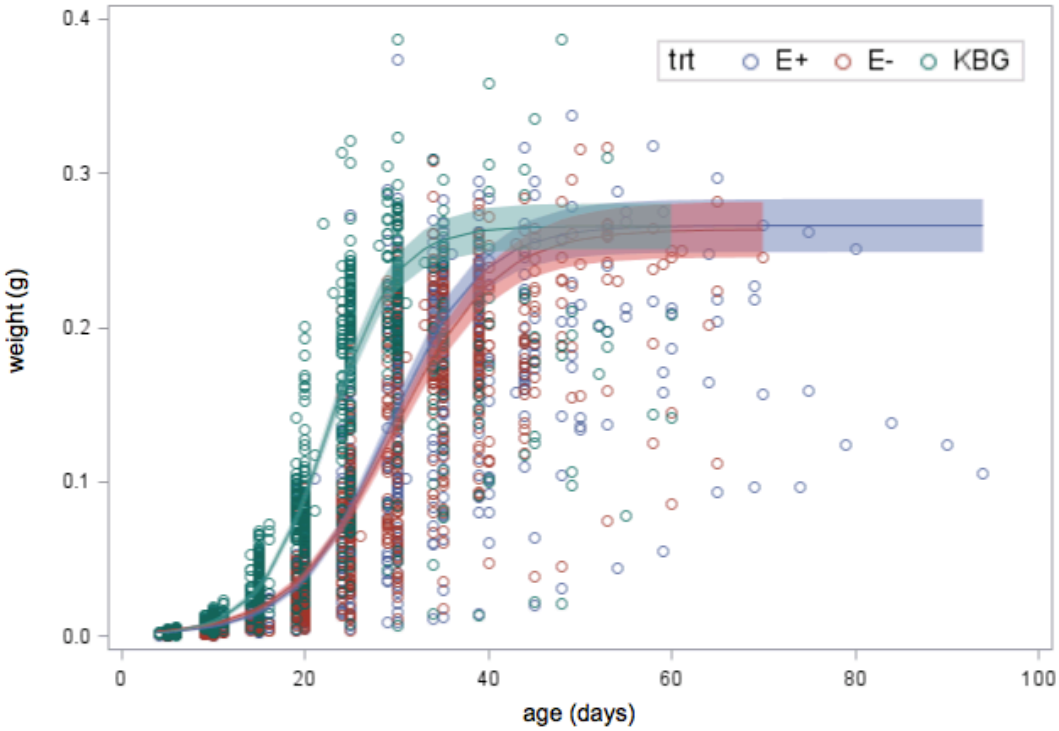

Figure 2. Estimated growth curve of larvae body weight over time for three different grass diets: Kentucky bluegrass (KBG), endophyte-infected tall fescue (E+), and endophyte-free tall fescue (E-).

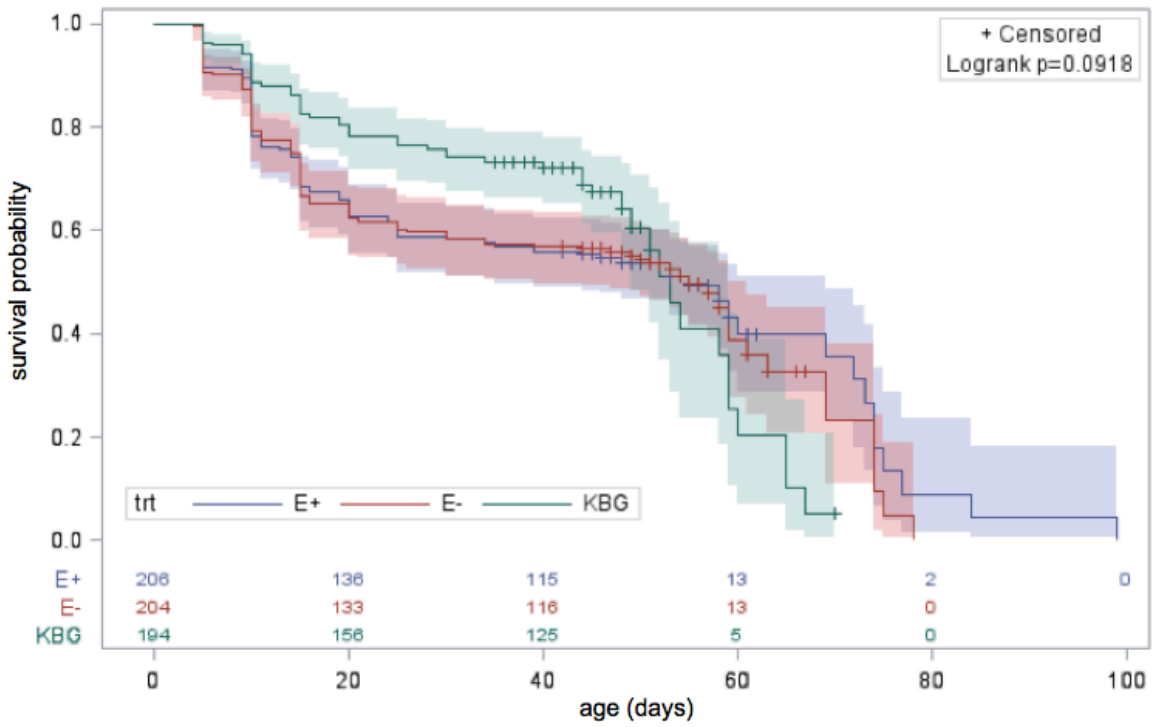

Figure 3. Kaplan Meier survival probability over time (days) for caterpillars feeding on three different diets: Kentucky bluegrass (KBG), endophyte-infected tall fescue (E+), and endophyte-free tall fescue (E-). The shaded areas represent 95% confidence limits. Values on the bottom of the graph represent the number of individuals remaining in each of the three treatments at any point in time. Individuals were considered censored if they developed normally, pupated, and eclosed as adults. A log rank test was used to compare the probability of survival to adulthood between the three treatments in the long-term.

CHAPTER 4. GENERAL CONCLUSIONS

The results within this thesis illustrate the ecologically complex role that tall fescue plays in grassland management. Previous studies of tall fescue have characterized the grass as an invasive-transformer – meaning that it can change the character, condition, form, and/or nature of ecosystems over substantial areas (Richardson et al. 2000, Tunnell et al. 2004). Our findings suggest that tall fescue is indeed degrading habitat and posing challenges for tallgrass prairie restoration, but the mechanisms that dictate its behavior are still unresolved.

Results from Chapter 2 show that patch-burn grazing management cannot substantially reduce tall fescue cover alone – herbicide use may be necessary to decrease tall fescue abundance. Additionally, the percentage of tall fescue cover did not significantly affect litter dynamics within our study pastures. Litter dynamics did fluctuate across experimental units within the patch-burn grazing pastures, but these changes were dependent on fire return intervals – not plant community composition. Patch-burn grazing management is predicted to create more heterogeneous habitat than either fire or grazing can create when applied alone (Fuhlendorf et al. 2009)., And indeed, we found patch-burn grazing treatment had the most heterogeneous litter depths within each year despite high levels of fescue cover. A mosaic of litter depths can be important for many insects and bird species (Vogel et al. 2007, Churchwell et al. 2008).

Our litter study in Chapter 2 could yield more useful results with a few improvements to the experimental design. I suggest future litter studies should include analyses of multiple classes of aboveground biomass rather than just fine surface litter collection; for example, it could be informative to measure differences in chemical composition (including alkaloid levels) in live biomass, standing dead material, and surface litter from each sample quadrat as

well as measuring before plant senescence. This method could illustrate nutrient cycling and decomposition differences across pastures with different fire and grazing treatments as well as distinct plant functional group characteristics. Taking this a step further, it would be valuable to measure soil organic matter (SOM) and soil nitrogen to explore how tall fescue dominance and endophyte-infection is affecting ecosystem processes like litter decomposition and soil nutrient availability. Research on belowground effects of endophyte-infected tall fescue is scarce (Saikkonen et al. 2015), and having a better understanding of these interacting factors could inform patterns of degradation and restoration pathways (McGranahan et al. 2013), especially as they relate to climate change (Bontti et al. 2009).

In Chapter 3 we examined the effect of endophyte-infected tall fescue on native insect forage quality. In the case of tawny-edged skippers, tall fescue, regardless of endophyte infection status, provided lower quality forage than Kentucky bluegrass (another exotic species). Interestingly, differences in alkaloid levels between uninfected and infected tall fescue did not result in major differences in survival or growth of the skippers in the lab. That said, Leuchtmann et al. (2000) found that loline alkaloid concentrations ranged from 0-2286 ppm in native European populations of tall fescue, and variability sometimes depended on age of the grass and endophyte morphology. Our samples contained a similar range of alkaloid concentrations in endophyte-infected samples (130 – 2292 ppm), but our grass plugs were only 4 – 8 weeks old. We suspect that endophyte infection and alkaloid concentrations may differ in a field situation because of plant maturity and/or environmental stresses, which could result in important differential effects on invertebrate herbivores. Further research on the fitness consequences of alkaloid consumption on additional native Lepidoptera seems warranted, particularly in light of the fact that many grass skipper species are in decline

(Schlicht et al. 2009, Swengel et al. 2011). In general, more knowledge is needed about the basic biology of many native grass skippers – including oviposition habitat selection in highly invaded landscapes. In particular, it would be valuable to rear individuals on a broader suite of native and exotic grasses that are common in degraded grasslands of the tallgrass prairie region.

Restoring degraded grassland habitat is not always straightforward – especially when invasive grasses like tall fescue have such unique and variable ecological effects (Johnson and Sandercock 2010, Hall et al. 2012, McGranahan et al. 2015). Though much research is still needed, our findings have important implications for grassland habitat managers. Exotic species composition matters for insect conservation, and, in order to improve biodiversity and ecosystem functioning, the use of different disturbance regimes such as a combination of herbicides, fire, and grazing may be needed to control invasive species like tall fescue.

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